

fast and efficient automated approach for microplastic polymer characterization, abundance numeration and size distribution. Moreover, a more detailed description of the particle was provided using the spatial information of the image to determine the size of MP particles and their frequency. In addition, HA is faster than the traditional library searching method, with results obtained in seconds once the models have been developed, improving the MPs throughput analysis.

3.09.T-10 Microplastics in Waste Water Treatment Plants: Monthly Analysis With FTIR and Py-Gc/Ms and Methodological Improvements

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Microplastics (MP) were shown to be present in various environments around the world. In order to better understand distribution patterns and to provide a solid basis for risk assessments of this organic pollutant, comprehensive datasets on MP concentrations and chemical compositions are needed. This study focussed on the effluents of two German wastewater treatment plants (WWTP) and investigated the temporal input of MP into the receiving river systems by performing a year-long sampling campaign with monthly sampling events. MP item data (minimum size: 11 µm) were generated using Fourier Transform Infrared (FTIR) spectroscopy, under the application of an improved polymer database. The database adaptation allowed for an improved data quality, as it counteracted matrix interferences due to residual plant material on measurement filters. Beside item data, complementary MP mass data were gained by the application of pyrolysis gas chromatography-mass spectrometry (Py-GC/MS) (for one WWTP). Both item and mass data showed homogeneous polymer compositions over the sampling year, generally dominated by polyolefins. Elevated MP item and mass concentrations occurred during winter months, and were accompanied by either heavy rainfall (resulting in increased discharge), total organic carbon or elevated turbidity values. These findings underline the necessity for the integration of background parameters in MP monitoring studies. Finally, by providing monthly data over one year on MP masses and items, this work contributes to the current knowledge on temporal MP dynamics in WWTP effluents, and can therefore be a useful baseline for future monitoring studies.

3.09 Micro- and nanoplastics: Towards the harmonized analysis for monitoring, effect studies and risk assessment (Part III)

3.09.T-11 Development of an Open-Source Hyperspectral Database and Model for Microplastics Classification and Comparison to Nile Red Staining

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Microplastics (MP) analysis is time-consuming, which limits our ability to monitor and respond to plastic pollution. Hyperspectral imaging is a rapid spectroscopic technique that can be used to image relatively larger surface areas than µFT-IR and Raman imaging systems. In this work, we develop a database and classification model for four polymer types (PP, PE, PET, PS) based on pristine, consumer product sourced and environmentally weathered plastics. A SIMCA chemometric model is used to classify hyperspectral images of spiked microplastic samples on glass fiber filters. Model performance parameters vary based on polymer type; however, specificity, sensitivity and accuracy are all greater than 85%. The quantification of MP sizes (area, Feret diameter, perimeter, circularity) is determined in parallel by HSI and Nile red staining with fluorescence microscopy to assess the performance of HSI. MP < 150 µm Feret diameter are not consistently detected by HSI when compared with results from Nile red staining; however, estimates for Feret diameter are consistent with Nile red staining for MP > 500 µm. HSI is shown to be a rapid method to accurately identify MP above 500 µm. With a simple user-friendly interface on an open-source application, this MP analysis workflow minimizes the need for expertise in spectroscopic methods enabling more researchers to rapidly carry out MP analysis. The workflow for analyzing hyperspectral images is open source and will be integrated into OpenSpecy, an existing database and analysis tool for MP spectral analysis. HSI could be a key technology for environmental monitoring frameworks where rapid imaging (< 30 seconds per sample) and analysis of multiple samples is required, however MP size ranges of interest must be above the limit of detection for the hyperspectral instrument being used.

3.09.T-12 Detection and Identification of Microplastics in Biota Using Nile Red and Machine Learning: Validation of an Innovative, Cost-Effective Approach

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Plastic pollution has become one of the most ubiquitous threats the oceans are facing nowadays. Microplastics (MPs) are of special concern as it has been shown these are ingested by a wide range of marine species from different trophic levels. However, the environmental implications of MPs ingestion, coupled to the toxicological relevance of different MP polymers and their chemical composition, remain poorly understood. This highlights the need for standardized, cost- and time-effective monitoring procedures to accurately and routinely determine the abundance, composition and distribution of MPs in the marine environment, to allow for effective management strategies. We developed an innovative approach for MP analysis in biota, thereby combining the advantages of both high-throughput screening and automation. We evaluated the method in mussel and different fish

gastrointestinal tracts (GIT) samples. The pretreatment involved a two-step digestion (10% KOH and 15% H₂O₂) at 50°C, with a stainless steel filtration step in between and an optional density separation step (NaI) to remove sediment, followed by filtration over a PTFE-filter and staining with the fluorescent dye Nile red (1 µg/ml acetone). MP detection and identification of the polymer types was done using two machine learning decision models. The first model predicted with high accuracy whether a particle was plastic or of natural origin. The second model allowed to identify the MP polymer type on the base of RGB colour data, which are extracted from Nile red-stained MPs, photographed through a fluorescence microscope under blue, green and UV filters. The efficiency and suitability of our approach was validated by spiking six MP types into the mussel and fish GITs, each one varying in the combination of polymer type (PAN, PET, PP, PS and PVC), size (250 – 1000 µm) and shape (particle/fibre). The validation took into account the accuracy, precision, limits of detection and quantification, selectivity, specificity, and robustness. This unique approach of high-throughput screening and machine learning automation, proved to be promising for the cost- and time-effective routine analysis of MPs in mussel and fish GITs in a simple, yet reliable way.

3.09.T-13 Characterization of Micro- and Nanoplastic Exposure in Human Tissues Using an Exposomic Framework

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Despite rapid advances in our understanding of micro- and nanoplastics (MNPs) in environmental matrices, significant data gaps hinder our understanding of MNP exposure in humans. Studies involving small sample sets investigating specific tissues have thus far established the presence of MNPs in human feces and placenta^{1,2}. Further work is required to more fully understand MNP exposure across a range of human tissues to deduce associated health outcomes. However, this goal is hindered by analytical challenges associated with the heterogeneous and complex nature of MNPs. Most environmental studies of MNPs focus on a few candidate polymers or chemicals that capture only a small fraction of MNP complexity. To overcome these and other limitations we have developed an untargeted HRMS analytical framework to characterize known and unknown MNPs, their monomers and additives, and related exposure biomarkers and biological response. This analytical framework combines 1) pyrolysis (GC) gas-chromatography with HRMS (Pyr-HRMS) to characterize MNP levels, 2) alkaline-assisted hydrolysis with liquid chromatography HRMS (AAH-HRMS) to measure particle monomers and additives, and 3) untargeted liquid chromatography with HRMS (LC-HRMS) to characterize the metabolome for the presence of MNP constituents and additives and biological response profiles. This framework was applied to placenta samples, virgin plastics, and weathered plastics to validate our approach. Pyr-HRMS detected a range of common MNP types in placenta tissue, including PS, PE and PMMA which were detected at a range of concentrations from 10-2650 ng/g. LC-HRMS revealed that free and particle-bound small molecule profiles were significantly different between virgin and weathered particles. Results to date validate the application of this framework to contemporaneously characterize polymers, free monomers, additives, and biomarkers of disease in human tissues; this framework provides an important toolset to expand our understanding of MNP exposure in humans and other biological receptors.

3.09.T-14 Quantification of NanoPlastics Association With Cells: Advances in Single-Cell Inductively Coupled Plasma Time of Flight Mass Spectrometry (sc-ICP-TOFMS) for Fast and Sensitive Detection of Metal Doped NanoPlastics

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Plastic pollution is a growing environmental issue and has triggered a need for appropriate hazard assessment strategies. Particulate plastic is present in the environment along a size continuum (macroplastics, microplastics and nanoplastics), and different size classes will dominate different fate and transport routes, as well as possibilities for biological interactions. Nano-sized plastics may pass through biological barriers and enter cells. Unfortunately, analytical tools for sensitive and quantitative detection of nano-sized plastics in cells and tissues are largely lacking. Indeed, the assessment of nanoplastics association with cells is more analytically challenging than other metal-based nanomaterials (e.g. engineered nanomaterials) considering that (nano)plastics chemical composition is more difficult to distinguish amidst high concentrations of organic matter with lower analytical detection limits. Therefore, assessing risks of (nano)plastic pollution demands creativity and requires innovative analytical methods, even in a laboratory context. An elegant approach is to dope nanoplastics with an inorganic tracer, thereby allowing for a more effective detection and tracking in complex matrices using common analytical techniques appropriate for metal analysis, such as ICP-MS. In this work, we synthesized nanoplastics particles with an embedded inorganic fingerprint (Pd) and used them to investigate the association of nanoplastics with human cells grown in culture using single-cell ICP-TOFMS. The simultaneous elemental detection of ICP-TOFMS enables the measurement of the full mass spectrum, thereby allowing the simultaneous detection of a cell elemental “fingerprint” together with the metal tag of the nanoplastics particles. Consequently, this method offers a direct quantitative measure of the nanoparticle-cell association. The potential for this approach is highlighted by analysing human cells (THP1 monocytes and A549 lung epithelial cells) exposed to low concentrations of Pd-doped nanoplastics (0, 5 and 50 mg/L; 24h). Additional challenges and improvements related to sample preparation, sample introduction and transport efficiency were also overcome to develop a robust and reliable method. Collectively, the study presents a strong foundation for a powerful analytical method to explore nanoplastics exposures in a variety of conditions, ranging from ecotoxicological studies to human health related questions.

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